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ANTIBACTERIAL EFFECTS OF *AZADIRACHTA INDICA* AND *CYMBOPOGON CITRATUS* AGAINST SOME COMMON HUMAN PATHOGENS.

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ABSTRACT: *Azadirachta indica and Cymbopogon citrates have been traditionally used for their therapeutic properties hence the study evaluated the anti-bacterial effects of these plants extracts against common human pathogens, including Escherichia coli, Salmonella typhi, Shigella spp. and Klebsiella pneumoniae. Ethanol extracts of plant samples were prepared using a wet extraction method. The antibacterial activity of the extracts was assessed using the agar well diffusion method. Clinical strains of sample organisms were used in susceptibility testing. The result varied with different ratios of solvents: all ethanol, 80:20 ethanol, 50:50 ethanol and all water. The study showed that both extracts have some level of antibacterial capacity. However, the control (Ciprofloxacin tablet) demonstrated high levels of sensitivity against all the test organism at higher level than the plant extracts. The study concluded that the yield of sample extracts varies significantly depending on the type of solvent used. It is suggested that Azadirachta indica concentrates have the potential to be used as an antibacterial agent against E. coli, Shigella spp, S. typhi, and K. pneumoniae and should be considered as potential alternative sources of antimicrobial agents.*

KEY WORDS: Azadirachta indica, Cymbopogon citrates, Human pathogens.

INTRODUCTION

Plants compounds and their derivatives are nature's gifts to humanity for their basic health requirements and sustenance (Akbar and Arshad, 2019; Hanifah, 2011). They have been used for millennia as a powerful medicinal treatment for a variety of illnesses and diseases (Faiz *et. al.*, 2017). Numerous bioactive substances found in plants, such as phenols, tannins, saponins, steroids, alkanoids, flavanoids, carbohydrates, and glycosides, are responsible for their antimicrobial effects and activities (Elisha, 2017; Batool, 2019).

Azadirachta indica (Neem leaf) belongs to the family Meliaceae has historically been used to treat ailments that purify the blood and skin. Neem's impact on the skin is possibly its most lauded benefit (Schmahl *et al.*, 2010). As general antiseptics, preparations made from the tree's leaves or oils are employed. Neem is effective in treating most epidermal disorders like acne, psoriasis, and eczema because of its antibacterial properties. Udeinya *et al.*, (2008) recorded Neem's analgesic, anti-inflammatory, and fever-lowering compounds that can be used to treat a variety of ailments, including cuts, burns, sprains, earaches, headaches, and fevers. Chaturvedi et. *al.*, (2011) reported numerous studies on the subject that have supported the use of neem extracts in the treatment of malaria. It is postulated that the flavonoid compounds of

neem leaves may be responsible for the anti-inflammatory activities in the body (Owoyele *et al*., 2016).

Azadirachtin, nimbin and nimbidine are the most abundance bioactive compounds found in the leaves of Neem however, the flowers, seeds, fruits, roots and bark of neem tree are also used for the treatment of infections on skin, teeth and gums. Kumar, 2009 reported the use of neem leaf extract in immunosuppressed birds which increased humoral and cell mediate immunity.

Cymbopogon citratus (Lemon Grass) belongs to Poaceae family can be used in treating HIV complications, especially secondary bacterial infections (Ekpenyong *et al*., 2015; Wright *et al*., 2009). Out of over 50 native grass species in the genus *Cymbopogon spp.* The most popularly used cultivar is ornamental lemon grass (*Cymbopogon citratus*), which is used frequently in Thai, Vietnamese, and Cambodian dishes. This plant with a lemon scent enhances the flavour of soups, curries, teas, and other alcoholic beverages (Spriha *et al*., 2021). Lemongrass oil has analgesic, antimicrobial, antiseptic, carminative, astringent, fungicidal bactericidal and antidepressant properties, making it one of the most versatile and health promoting essential oils, which can help to kill both internal and external bacterial and fungal infections, such as ringworm and athlete's foot disease (Schweitzer *et al*., 2022) Lemongrass ranked highest in inhibitory activity against methicillin-resistant Staphylococcus aureus (MRSA) infection. It is also helpful in relieving colitis indigestion and gastro-enteritis ailments.

The effectiveness of lemon grass plant extract on the analgesic properties was reported to be approximately similar to the pentazocine drug an opioid pain medication which is sometimes referred to as a narcotic This drug also works as an analgesic which eventually interacts with k receptors which in turn causes sedation (Maryam Hassan *et al*., 2023).

It has been determined that certain plants, including *Azadirachta indica* (Neem leaf) and *Cymbopogan citrates* (lemon grass) have a variety of culinary and medicinal qualities that have bacteriostasis and bacteriocidal effects on some bacteria (Kusuma, e*t al.*, 2024). The natural antioxidants present in the neem leaf and lemon grass such as polyphenols play an important role in preventing the body from oxidative damage (Rao *et al.*, 2017). This is because the ideal chemical structure of polyphenols compounds makes the plants more effective as an antioxidant against a free radical scavenging activity as compared to any other compounds such as ascorbate and tocopherols (Rao *et al*., 2017).

Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, and *Shigella spp.,* are *s*ome common human pathogens that have been linked to diarrhea and other diseases or infections (Lockhart *et. al.*, 2007). *E. coli* produces a toxin that damages the lining of the small intestine, resulting in bloody diarrhea (Mueller and Tainter, 2023). Its diarrhea is characterized by copious amounts of watery diarrhea with little or no fever, and the stool yields a nearly pure *E coli* culture. *Salmonella typhi* causes diarrhea by invading epithelial cells and causing the release of proinflammatory cytokines, resulting in an inflammatory response.

Klebsiella pneumoniae is a harmless bacterium found in human intestines. If *K. pneumoniae* enters other parts of the body, it can cause pneumonia, bloodstream infections, meningitis, and urinary tract infections (Abbas *et al.*, 2024).

Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, and Shigella spp., are *s*ome common human pathogens that have been tried by various researchers globally to inhibit its growth and prevalence using various medicinal plant parts extracts. Limited reports are

available on comparison of the antibacterial effects of indigenous medicinal plants (*Azadirachta indica* (Neem leaf) and *Cymbopogan citrates* (lemon grass)) on some common human pathogens (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae,* and *Shigella spp.)* thus the need for this study to assess the antimicrobial effect of these plants extracts of different controlled concentrations on the selected human patogens.

2. MATERIALS AND MEHODOLOGY

Collection and Processing of plant samples

Fresh leaves of the *Azadirachta indica* (Neem leaf) and *Cymbopogon citratus* (lemon grass) were collected from Iwo in Isale-Oba Compound, Iwo, Osun State. The plant leaves were washed in running water to remove adhesive contaminants, were dried in shade at room temperature for 10 days, and ground into powdered form, which was kept in an air tight container before further processing.

Source of micro organism

The bacterial isolates *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Shigella spp* used in this study were obtained from International Institute of Tropical Agriculture (IITA) Ibadan Oyo State Nigeria.

Preparation and Concentration of Extracts

To obtain different ethanolic concentration of leaf extract, 50 grams of *Azadirachta indica* (Neem leaf) and *Cymbopogon citratus* (lemon grass) were weighed into conical flask and 100 ml of extractant solvent (water and ethanol) were added and soaked at room temperature for 72 hours (3days) (Wylie *et al*., 2022).

Table 1: Composition of Concentrate

Concentrate	Composition
1	Ethanol
2	80% ethanol and 20% distilled water
3	50% ethanol and 50% distilled water
4	Distilled water

This extraction was done using maceration or wet method during which extract solutions were filtered aseptically into another different sample bottles using a sterile filter paper (Watt-man). The filtrates were placed into an evaporator at room temperature to drive-off the extractants and stored in refrigerator until needed for further use

Concentration of Extracts

The total weight for Azadirachta *indica* (Neem leaf) and *Cymbopogon citratus* (lemon grass) was 50g.

Concentration = $\frac{Weight\ of\ Dry\ leaf}{Volume\ of\ Extract\ Solvent}$ gives the concentration

Standardization and Preparation Of Innoculum

The test organism are *E.coli, S.typhi, K.pneumoniae* and *S.spp* which are all gram negative bacterial. The organism were activated by culturing in an fresh Nutrient Agar plates.

Mcfarland standard for test organism was prepared in test tubes by mixing 0.5ml of 1% BaCl (Barium Chloride) with 9.95ml of 1% H_2SO_4 to get approximate bacteria suspension Approximately 1.5 x 10^8 cfu/ml

Preparation Of Antibiotics (Control)

Ten 500mg of (Citab) Ciprofloxacin tablet was diluted into 100ml of sterile distilled water to obtain Ciprofloxacin diluents of 0.05g/ml which is equivalent to the minimum concentration of our extracts.

Antibiotics Concentration = $\frac{Weight\ of\ Tablets}{Volume\ of\ diluents}$ = $\frac{5000mg}{100ml}$ = 50mg/ml = 0.05mg/ml

Test for Antibacterial Activity of The Extracts

Antibiotics susceptibility was done using well boring method (Kerk bower method) and Spread plate method was used to introduced the inoculum. Sterile Mueller-hinton agar was prepared according to manufacturers standard, 15ml of cool molten agar was then dispense into different plates and allow to set. A sterile swab stick was dipped into standard inoculum and was used to rub the surface of the solidified agar plates, the plates were incubated for 10-15 minutes. After 15 minutes, the plate was brought out from incubator. A sterile cock borer of 6mm diameter was used to make one ditch on each inoculated plate and 0.5ml of each extract (ethanolic and aqueous extract) of concentrates 1, 2, 3, 4 was introduced into ditched wells. In addition, 0.5ml of concentrated antibiotic diluents was also introduced into inoculated plates well accordingly, and plates were labelled appropriately. After 24 hours of incubation, zone of inhibition was measured using millimeter rule.

3. RESULT

Table 2: Percent yield of Neem Leaf (*Azadirachta indica*) and Lemon grass (*Cymbopogon citratus*) using Ethanol and Aqueous Extract

Concentrations	Neem Leaf (*Azadirachta indica*)	Lemon grass (*Cymbopogon citratus*)
All ethanol yielded	0.09g/ml	0.09g/ml
80% ethanol and 20% water yielded	0.1g/ml	0.06g/ml
50% ethanol and 50% water yielded	0.03g/ml	0.05g/ml
Only water yielded	0.09g/ml	0.06g/ml

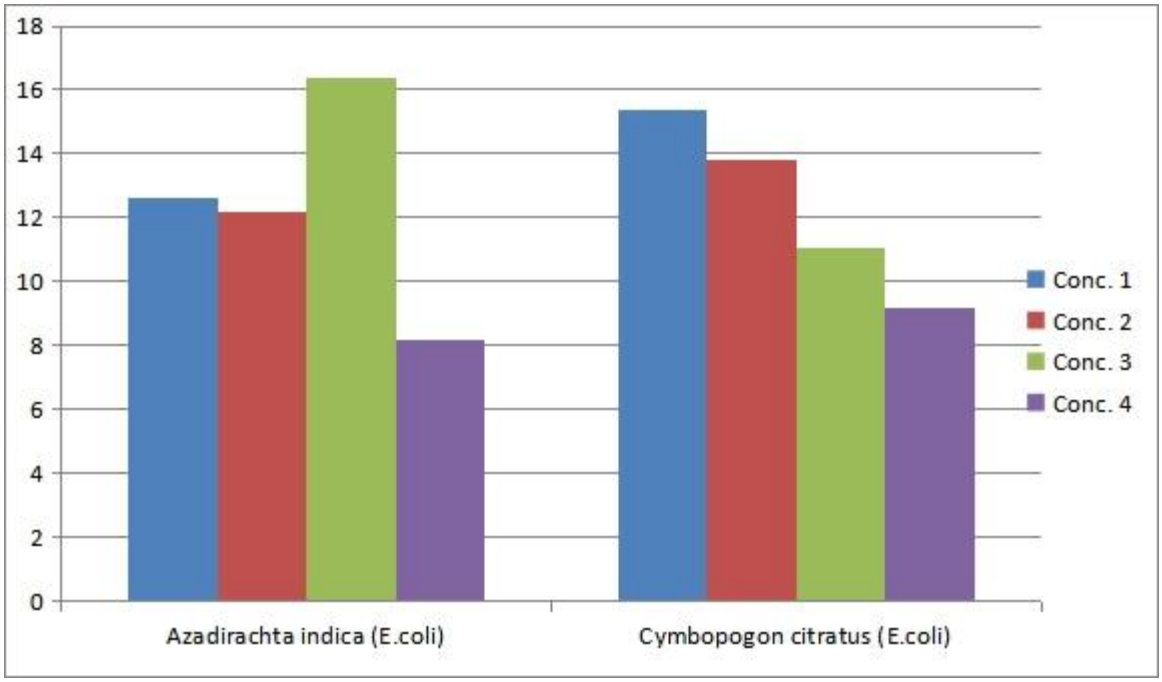

Figure 1: Comparative effect of different concentrate of *(Azadirachta indica)* and (*Cymbopogon citrates)* on *E. coli* strain

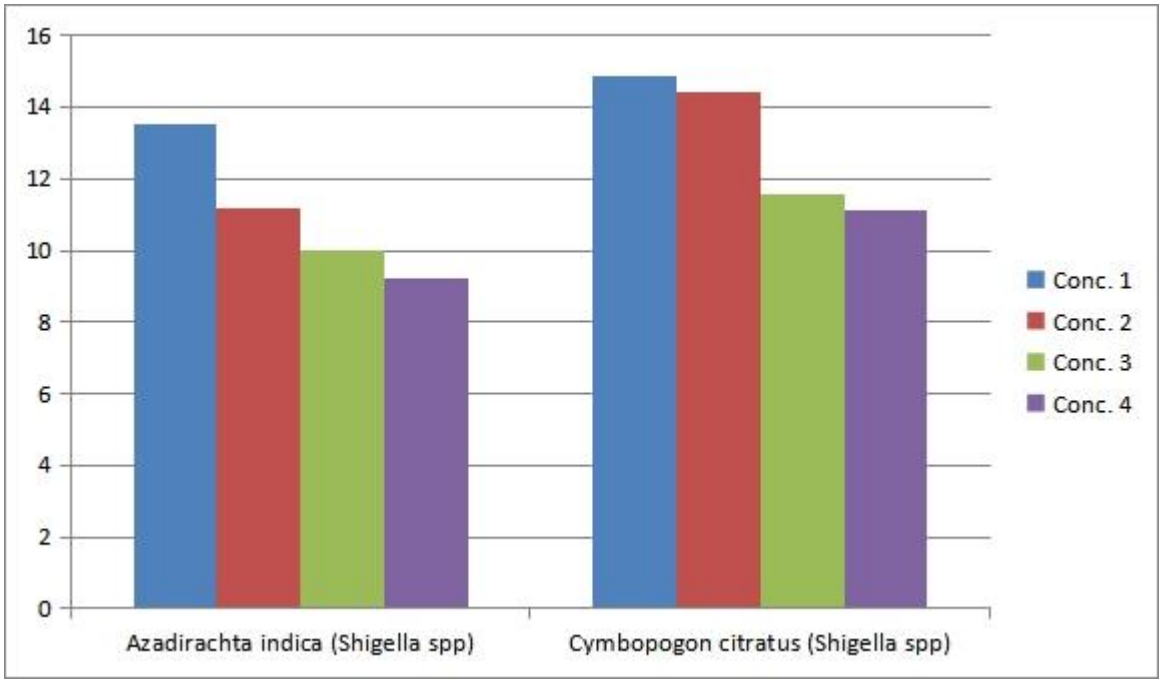

Figure 2: Comparative effect of different concentrate of *(Azadirachta indica)* and (*Cymbopogon citrates)* on *Shigella spp.* strain

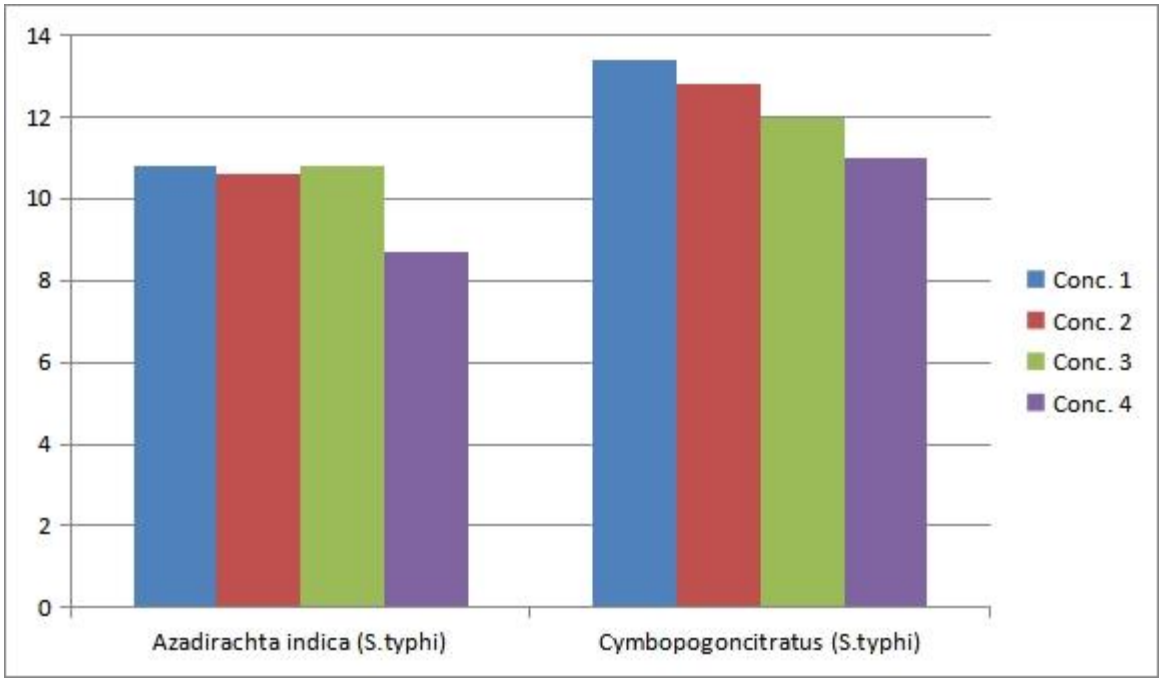

Figure 3: Comparative effect of different concentrate of *(Azadirachta indica)* and (*Cymbopogon citrates)* on *S. typhi* strain

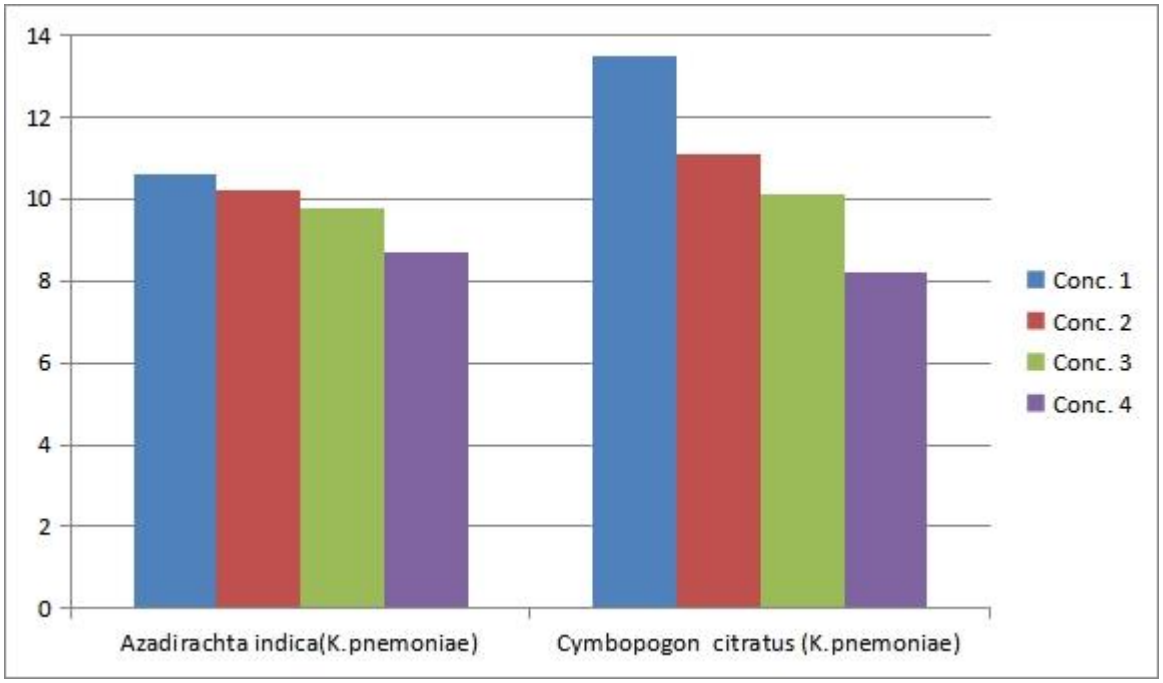

Figure 4: Comparative effect of different concentrate of *(Azadirachta indica)* and (*Cymbopogon citrates)* on *K. pnemoniae* strain

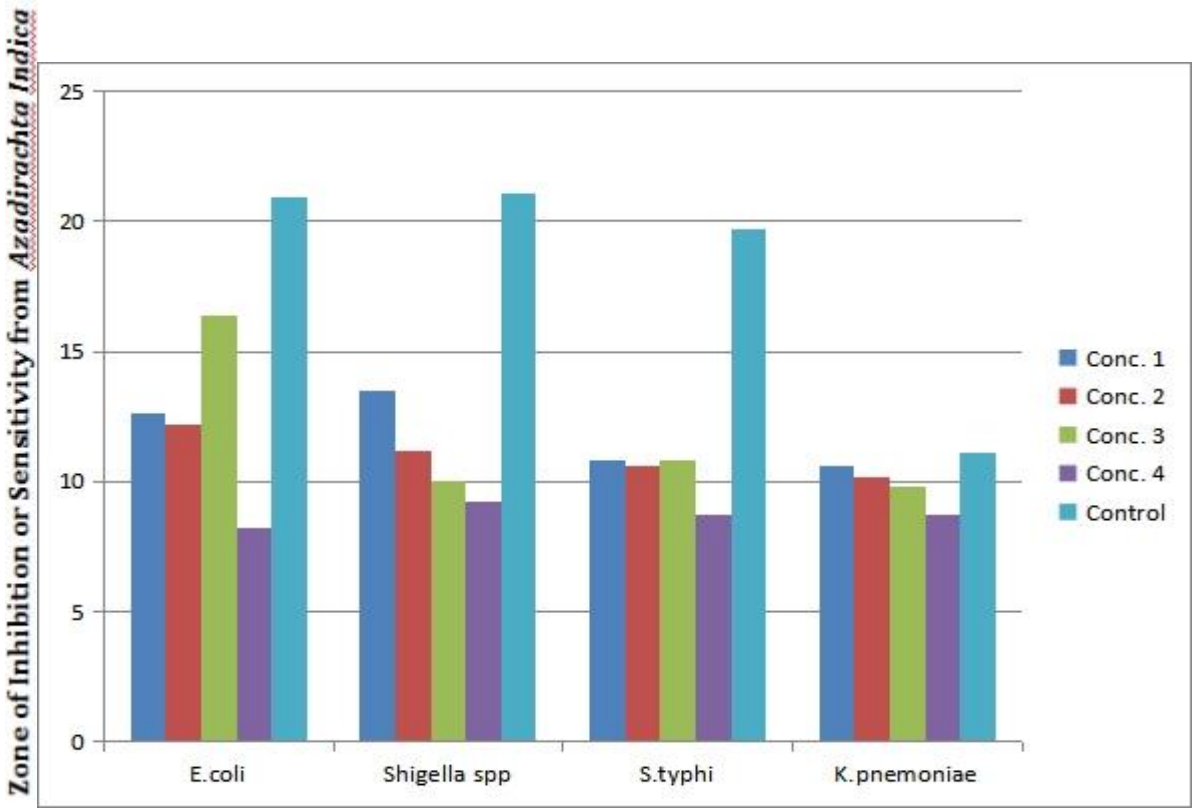

Figure 5: Comparing the Level of Inhibition Exhibited by Different Species of Human Bacteria Pathogen

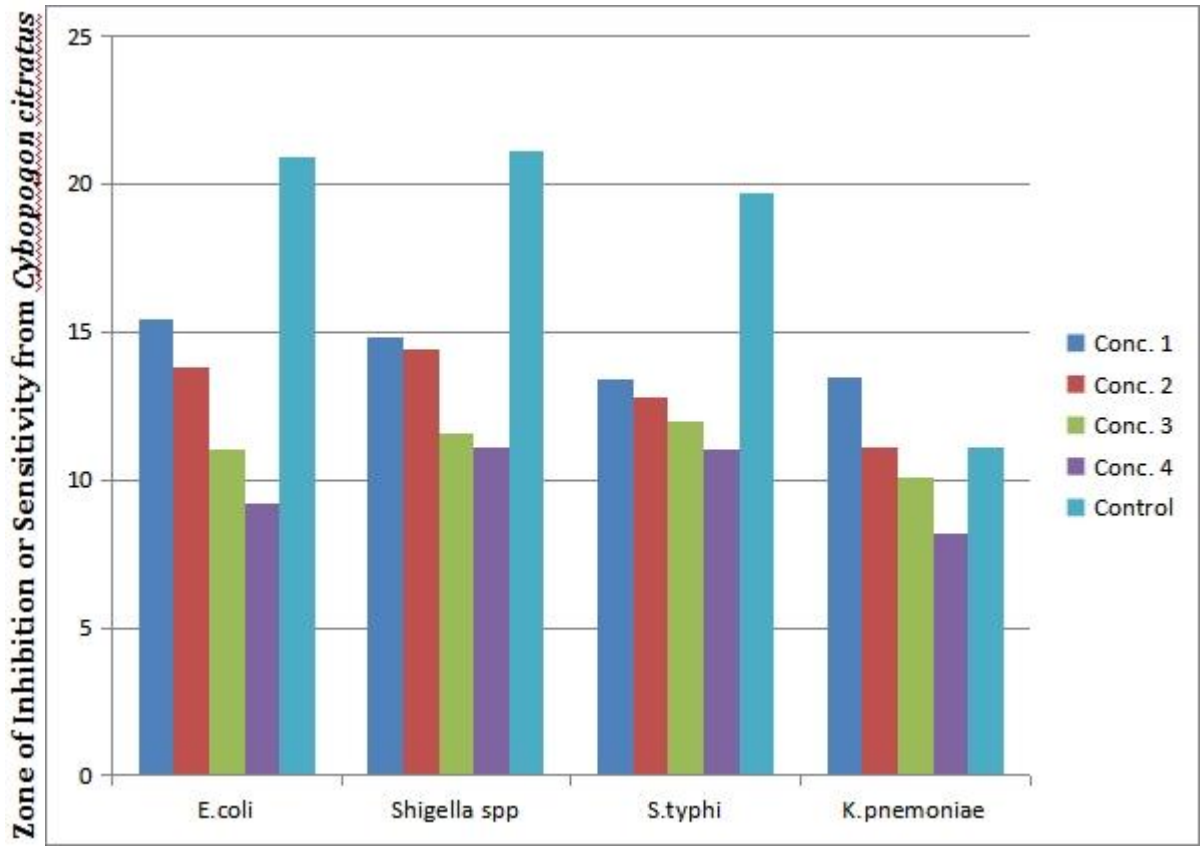

Figure 6: Comparing the Level of Inhibition Exhibited by Different Species of Human Bacteria Pathogen

4.2 DISCUSSION

From Table 2, it shows the percentage yield of extracts obtained from neem leaf (*Azadirachta indica*) and lemon grass (*Cymbopogon citratus*) using different solvents. The percentage yield of the extracts varied depending on the solvent used. The highest yield for neem leaf was obtained using 80% ethanol and 20% water, while the highest yield for lemon grass was obtained using 100% ethanol. The lowest yield for both neem leaf and lemon grass was obtained using 50% ethanol and 50% water.

Figure 1 indicates that both plant extracts have antimicrobial activity against E. coli strains. *Cymbopogon citratus* showed a consistently higher zone of inhibition than *Azadirachta indica* against all four strains of *E. coli.* Cymbopogon citratus may have stronger antimicrobial activity against *E. coli* than *Azadirachta indica.*

Figure 2 indicates that both plants extract have antimicrobial activity against *Shigella spp*. The zone of inhibition for both extracts was highest against *Shigella spp*. I and lowest against *Shigella sp.* IV. Figure 2, comparing the two plant extracts, *Cymbopogon citratus* showed a consistently higher zone of inhibition than *Azadirachta indica* against all four strains of *Shigella spp*. This suggests that *Cymbopogon citratus* may have stronger antimicrobial activity against *Shigella spp* than Azadirachta indica. *Shigella spp* is a Gram-negative bacterium that causes Shigellosis, an infectious disease characterized by diarrhea, fever, and abdominal cramps. The results of this study suggest that both *Azadirachta indica* and *Cymbopogon citratus* have the potential to be used as natural antimicrobial agents against *Shigella spp*.

Figure 3, indicates that both plant extracts have antimicrobial activity against S. typhi. *Cymbopogon citratus* showed a consistently higher zone of inhibition than *Azadirachta indica. S.typhi* is a Gram-negative bacterium that causes typhoid fever, a potentially life-threatening illness. The results of this study suggest that both *Azadirachta indica* and *Cymbopogon citratus* have the potential to be used as natural antimicrobial agents against *S. typhi.*

Figure 4, indicates that both plant extracts have antimicrobial activity against *K. pneumoniae. Cymbopogon citratus* showed a higher zone of inhibition than *Azadirachta indica* against *K. pneumoniae* I and III, while *Azadirachta indica* showed a slightly higher zone of inhibition against *K. pneumoniae* II and IV. However, the overall difference in zone of inhibition between the two extracts was not as pronounced as in the previous values. *K. pneumoniae* is a Gram-negative bacterium that can cause pneumonia, urinary tract infections, and other infections. The results of this study suggest that both *Azadirachta indica* and *Cymbopogon citratus* have some level of antimicrobial activity against *K. pneumoniae.* It is worthy to note that the concentration and purity of the plant extracts used may also affect their antimicrobial activity (Kalidindi *et al.,* 2015).

Figure 5 show that the zone of inhibition varied depending on the concentration of the neem leaf extract. The highest average zone of inhibition was obtained at concentration 3 for E. coli with a value of 16.4 mm. The highest average zone of inhibition for *Shigella spp* was obtained at concentration 1 with a value of 13.5 mm. For *S. typhi,* the highest average zone of inhibition was obtained at concentration 1 with a value of 10.8 mm. For *K. pneumoniae,* the highest average zone of inhibition was obtained at concentration 1 with a value of 10.6 mm.

In figure 6, the control group had the highest zone of inhibition for all bacterial strains except for *K. pneumoniae*. This indicates that the neem leaf extract was less effective at inhibiting the

growth of the bacteria compared to the control group. The results suggest that the neem leaf extract has antimicrobial properties against the tested bacterial strains, with varying levels of effectiveness depending on the bacterial strain and concentration of the extract. The highest concentration of the extract (concentration 3) was the most effective against *E. coli*, while the lowest concentration (concentration 4) was the least effective against all bacterial strains tested. The results suggest that neem leaf extract may have potential as a natural antimicrobial agent, but further studies are needed to identify the specific compounds responsible for the observed effects and to determine their mechanism of action

Figure 6 show that the zone of inhibition varied depending on the concentration of the lemongrass extract. The highest average zone of inhibition was obtained at concentrate 1 for *E. coli* with a value of 15.4 mm. The highest average zone of inhibition for *Shigella spp* was obtained at concentrate 1 with a value of 14.85 mm. For S. typhi, the highest average zone of inhibition was obtained at concentrate 1 with a value of 13.4 mm. For *K. pneumoniae*, the highest average zone of inhibition was obtained at concentrate 1 with a value of 13.5 mm. As shown in figure 6, the control group had the highest zone of inhibition for all bacterial strains except for *K. pneumoniae.* This indicates that the lemongrass extract was less effective at inhibiting the growth of the bacteria compared to the control group.

The results suggest that the lemongrass extract has antimicrobial properties against the tested bacterial strains, with varying levels of effectiveness depending on the bacterial strain and concentration of the extract. Which is inline with the findings of Chollom *et. al.,*2022 and Lockart *et al.,* (2007) who worked on similar human pathogens and plant extracts. The highest concentration of the extract (concentrate 1) was the most effective against all bacterial strains tested, while the lowest concentration (concentrate 4) was the least effective against all bacterial strains tested.

5. CONCLUSION AND RECOMMENDATION

Extraction was solvent sensitive with 80% ethanolic solvent more efficient in extraction of *Azadirachta indica* and 100% etthanolic solvent more effective for extraction of *Cymbopogon citrates,* while extraction yield better extract for *Azadirachta indica*. The study found that the extracts obtained from *Azadirachta indica* and Cymbopogon *citratus* demonstrated some level of inhibition effects on the growth of common human pathogens used and therefore may be useful in treating infections or diseases linked to them.

It is recommended to still utilize the god given plants studied in the treatment of infections at accurate portions which could always be pocket friendly than the control (Ciprofloxacin tablet) demonstrated high levels of sensitivity against test organisms. Also, the therapeutic potentials in these plants should be explored further beyond antibacterial activities.

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